University of California Office of the President
Special Research Programs

Annual Progress Report
SUMMARY OF SCIENTIFIC PROGRESS
Form 3

(Check one) [X] Breast Cancer Research [ ] Tobacco-Related Disease Research [ ] Universitywide AIDS Research

AWARD NUMBER: 4JB-0089 PROJECT YEAR *(Check one)*: [ ] 1st [ ] 2nd [ ] 3rd [X] Final

PRINCIPAL INVESTIGATOR(S): Paul D. Kaufman

PROJECT TITLE: Regulation of Breast Cell Lifespan by a Chromatin Protein

INSTITUTION: E.O. Lawrence Berkeley National Laboratory (LBNL)

The summary must include:

a) A statement of each specific aim, followed by an account of progress made towards its accomplishment, including a summary of experimental results.
b) If an original aim was dropped or modified, an explanation of the reason for such a change.
c) If a new aim was added, progress toward its achievement.
d) The relevance of any modified or new specific aim(s) to the project's mission and research priorities

Do not exceed 5 pages; number any additional pages as 3a, 3b, etc.

(Collaborative Grants should submit one combined progress report.)

(See Sections 7.3 and 7.4 for special TRDRP and UARP reporting requirements.)

1. SPECIFIC AIMS

1. Overproduce dominant-negative CAF-I protein fragments (p150C) using recombinant retroviruses.

2. Determine the effects of CAF-I perturbation on breast fibroblast and epithelial cells with respect to gene expression and cellular lifespan.

3. Determine the effects of CAF-I perturbation on aspects of chromosome structure implicated in cellular senescence and tumorigenesis: telomere length, nucleolar structure and DNA repair.

2. Changes in specific aims: None.

3. Research progress

Previously, we demonstrated that we can produce the p150C protein fragment in human cells. This was done by retroviral-mediated gene transfer into human diploid fibroblasts followed by drug selection. However, the initial population of cells proved to have only modestly reduced levels of endogenous CAF-I subunits. Also, these cells did not display significant growth differences or reduced ability to repair ultraviolet radiation damage (data not shown). Therefore, our next approach was to isolate clones from the initial transformed population that express higher levels of p150C. To do this, we isolated clones by limiting dilution and simultaneously put them under stronger selection for p150C expression by increasing the selective drug concentration. The isolated cloned lines fell into two classes. One class was able to grow nearly as rapidly as the starting population and displayed little difference in endogenous CAF-I levels, and was therefore not studied further. The other class initially grew much more slowly, with a doubling time approximately one-half that of the parental population. Significantly, members of this class displayed a significant reduction in endogenous CAF-I levels. However, none of the slow-growing cell lines could be maintained in culture. Therefore, continuous overexpression of p150C appeared to be incompatible with cell survival.

We therefore wished to confirm our notion that p150C was indeed able to inhibit CAF-I activity, and if so devise alternative strategies for its use as an inhibitor *in vivo*. First, we demonstrated that *in vitro*-translated p150C would indeed inhibit the activity of human CAF-I *in vitro* (Figure 1). Then, in collaboration with the laboratory of Dr. Peter Adams at the Fox Chase Cancer Center in Philadelphia, PA, we demonstrated that transient expression of p150C arrests cells in S phase (Figure 2). This dramatic block to cell cycle progression is consistent with our notion that continual inhibition of CAF-I blocks growth of human cells. Furthermore, these data suggest that a checkpoint operates in human cells that ensures coupling between DNA synthesis and histone deposition. We are currently more finely mapping the region of p150C required for both inhibition of CAF-I activity and S phase arrest. We are especially interested in determining if any of these activities correlate with the known protein-protein interactions in this region (p60 binding and p150 dimerization), which we are also continuing to map.

The C-terminus of p150 inhibits CAF-I activity *in vitro*

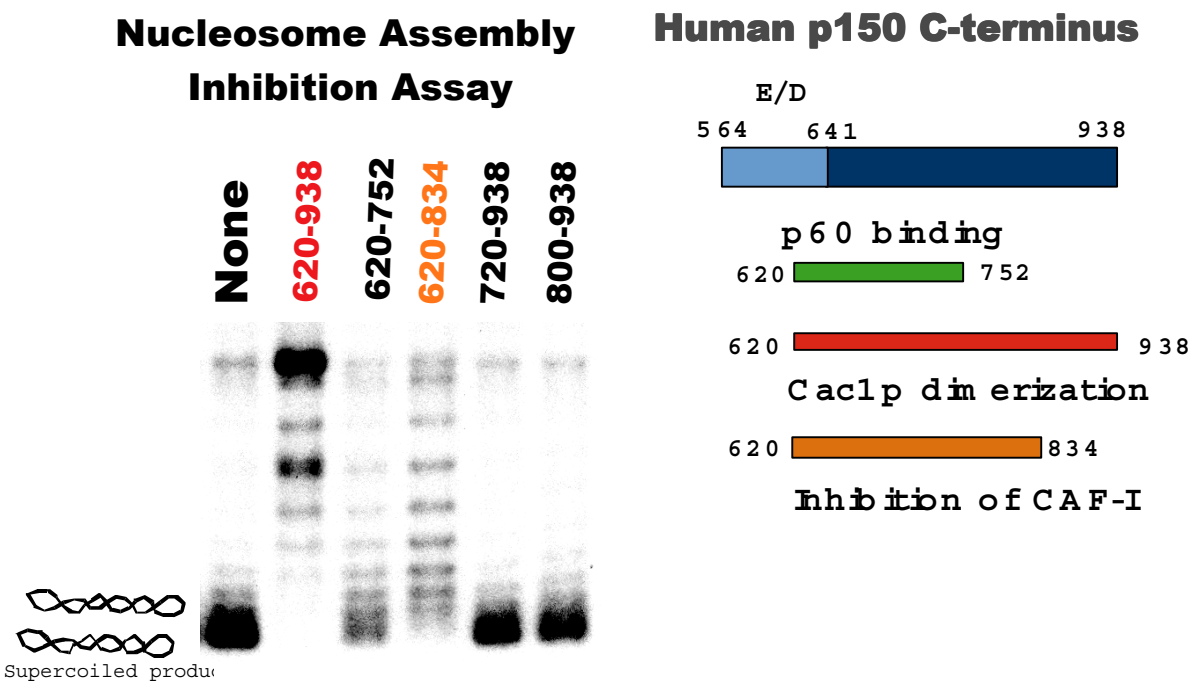

Figure 1. The C-terminus of CAF-I p150 inhibits nucleosome assembly *in vitro*. On the left, nucleosome assembly during an *in vitro* SV40 DNA replication was stimulated by purified human p150. Nucleosome assembly is indicated by formation of negatively supercoiled DNA species at the bottom of the gel. *In vitro* translated fragments of p150 were added as indicated above the figure. On the right is a diagram of the human p150 C-terminal residues and regions required for known protein interactions or functions. Interestingly, the regions that inhibits CAF-I function also is able to generate an S phase arrest (see Figure 2).

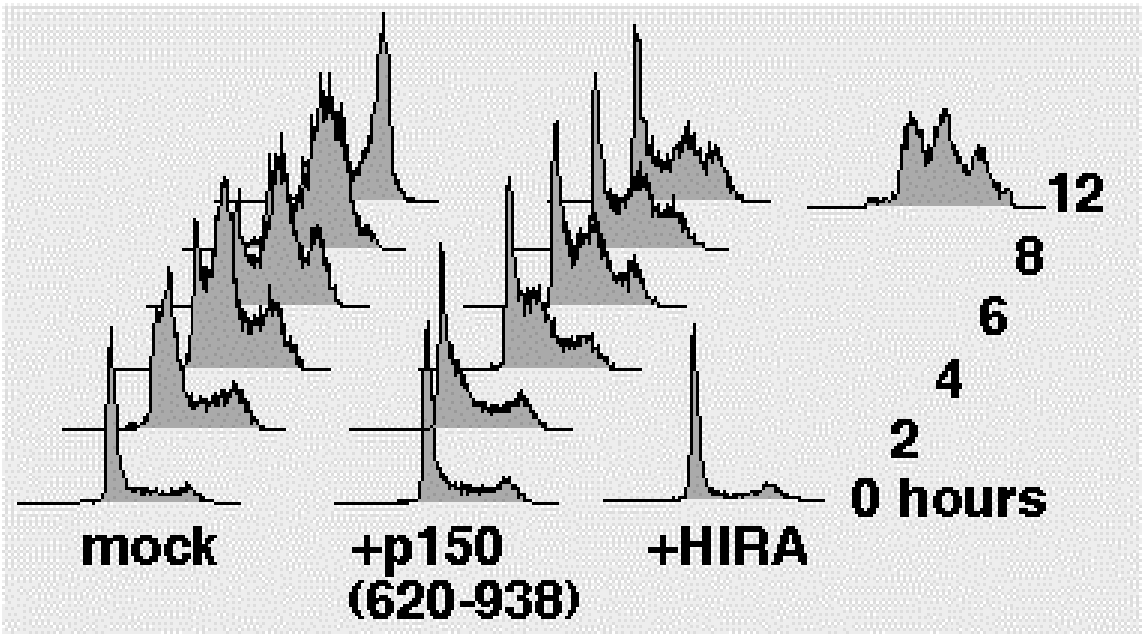

Figure 2. p150C blocks DNA synthesis in vivo. Asynchronously growing U2OS cells were transiently transfected with the cell surface marker CD19 plasmids encoding p150C, an empty vector as mock, or the human HIRA protein know to induce S phase delays. Cells were arrested in hydroxyurea and released into drug-free media to allow progression through S phase. Cells were harvested at the indicated time points and CD19-positive cells were analyzed for DNA content by fluorescence-activated cell sorting.